# On the osteogenic expression induced by calcium/phosphate deposition

**To the Editor**: In a recently published paper in this journal, Professor Demer's group[1] suggests that calcium/phosphate crystals (hydroxyapatite) induce the expression of osteogenic genes, *Bmp2* and *Osteopontin*, in vascular smooth muscle cells. This conclusion agrees with another recent paper from our group.[2]

Calcium/phosphate-induced calcification is a process of hydroxyapatite deposition that is initially independent of any cell activity or metabolism.[2] The initial step consists of passive calcium/phosphate deposition, in which the role of calcium is more prominent than that of phosphate,[2,3] according to recent studies by both Professor Shanahan's group[3] and our group.[2] The reason for this is that a high concentration of phosphate (4 mmol/l) in the presence of a low concentration of calcium (1 mmol/l) does not produce calcium/phosphate deposition or upregulation in the expression of osteogenic genes.[2] By contrast, high concentrations of calcium (4 mmol/l) in the presence of 1 mmol/l phosphate induces both the formation of calcium/phosphate crystals and upregulation in the expression of osteogenic genes *Cbfa1* and *Bmp2* (Figure 1).[2]

Although it was previously accepted that the expression of osteogenes induced the formation of hydroxyapatite deposits, all these studies now show that calcium/phosphate deposition is indeed responsible for osteogene expression under hyperphosphatemic conditions. Moreover, a cell attempts to prevent these deposits by producing inhibitors.[2] These results are taking another look at the role of phosphate and related Pi transport inside the cell during the calcification process.[4]

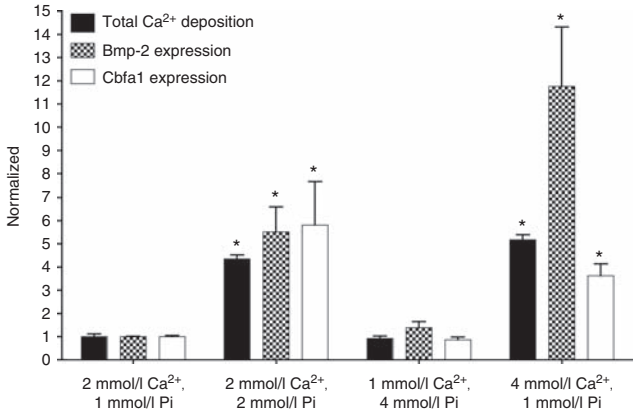


**Figure 1 | Comparison of total calcium deposition, and Bmp-2 and Cbfa1 expressions in vascular smooth muscle cells after 3 days of incubation with the indicated concentrations of calcium and phosphate in MEM culture medium.** *Statistically different from the 2 mmol/l $Ca^{2+}$, 1 mmol/l Pi condition.


1. Sage AP, Lu J, Tintut Y *et al.* Hyperphosphatemia-induced nanocrystals upregulate the expression of bone morphogenetic protein-2 and osteopontin genes in mouse smooth muscle cells *in vitro*. *Kidney Int* 2011; **79**: 414–422.
2. Villa-Bellosta R, Millan A, Sorribas V. Role of calcium-phosphate deposition in vascular smooth muscle cell calcification. *Am J Physiol Cell Physiol* 2010; **300**: C210–C220.
3. Shroff RC, McNair R, Skepper JN *et al.* Chronic mineral dysregulation promotes vascular smooth muscle cell adaptation and extracellular matrix calcification. *J Am Soc Nephrol* 2010; **21**: 103–112.
4. Villa-Bellosta R, Bogaert YE, Levi M *et al.* Characterization of phosphate transport in rat vascular smooth muscle cells: implications for vascular calcification. *Arterioscler Thromb Vasc Biol* 2007; **27**: 1030–1036.



Ricardo Villa-Bellosta[1] and Víctor Sorribas[1]

[1]*Laboratory of Molecular Toxicology, University of Zaragoza, Zaragoza, Spain*

**Correspondence:** *Ricardo Villa-Bellosta or Víctor Sorribas, Laboratory of Molecular Toxicology, University of Zaragoza, Calle Miguel Servet 177, 50013 Zaragoza, Spain. E-mail: rvilla@unizar.es or sorribas@unizar.es*


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**The Authors Reply:** We appreciate the comments from Sorribas and Villa-Bellosta[1] and are pleased to see that Professor Sorribas and colleagues[2] have simultaneously and independently made observations similar to ours:[3] that, under hyperphosphatemic conditions *in vitro*, calcium phosphate nanocrystal formation can be initiated independently of cells. Both sets of findings introduce a novel, alternative mechanism for osteogenic gene induction and further support the concept that the stages of vascular calcification, much like those of bone mineralization, are actively regulated. Thus, depending on the underlying clinical or culture conditions, mineralization may be governed by a wide variety of nucleators, inhibitors, and activators as detailed by George and Veis.[4]


1. Villa-Bellosta R, Sorribas V. On the osteogenic expression induced by calcium/phosphate deposition. *Kidney Int* 2011; **79**: 921.
2. Villa-Bellosta R, Millan A, Sorribas V. Role of calcium-phosphate deposition in vascular smooth muscle cell calcification. *Am J Physiol Cell Physiol* 2011; **300**: C210–C220.
3. Sage AP, Lu J, Tintut Y *et al.* Hyperphosphatemia-induced nanocrystals upregulate the expression of bone morphogenetic protein-2 and osteopontin genes in mouse smooth muscle cells *in vitro*. *Kidney Int* 2011; **79**: 414–422.
4. George A, Veis A. Phosphorylated proteins and control over apatite nucleation, crystal growth, and inhibition. *Chem Rev* 2008; **108**: 4670–4693.



Andrew P. Sage[1], Yin Tintut[2] and Linda L. Demer[2,3,4]

[1]*Division of Cardiovascular Medicine, University of Cambridge, Addenbrookes Hospital, Cambridge, UK;* [2]*Department of Medicine, UCLA, Los Angeles, California, USA;* [3]*Department of Physiology, UCLA, Los Angeles, California, USA and* [4]*Department of Bioengineering, UCLA, Los Angeles, California, USA*

**Correspondence:** *Linda L. Demer, Division of Cardiology, David Geffen School of Medicine at UCLA, 10833 LeConte Avenue, Box 951679, Los Angeles, California 90095-1679, USA. E-mail: LDemer@mednet.ucla.edu*


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